Supplementary Materials for

Interferon Resistance of Emerging SARS-CoV-2 Variants

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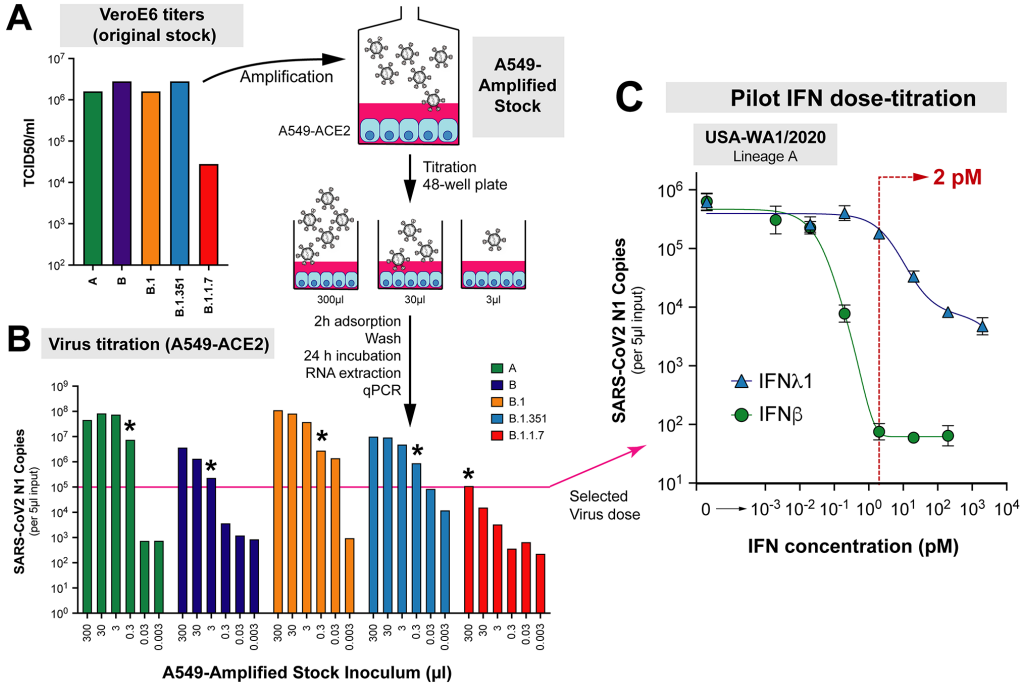
This PDF file includes:

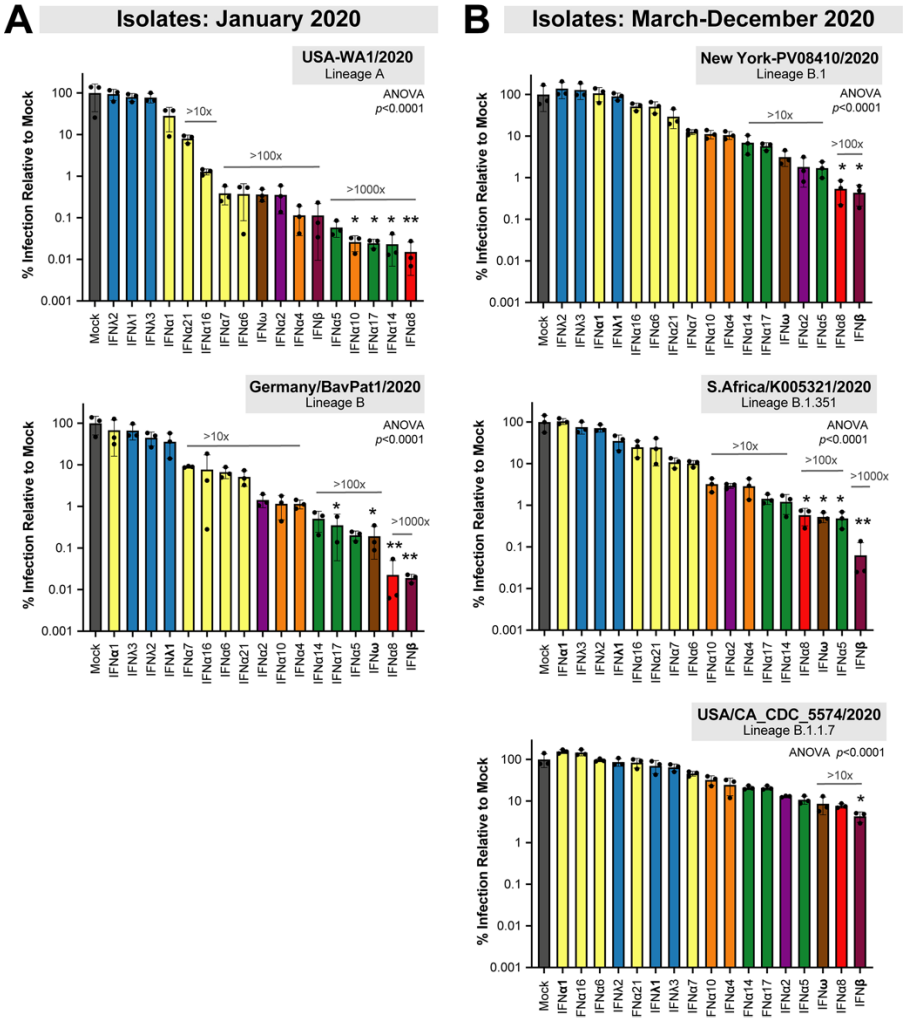
Supplementary Table 1. SARS-CoV-2 Isolates Tested for IFN-I and IFN-III sensitivity.

BEI Catalogue Number	Isolate Name	Collection Date	GISAID clade	Lineage	GISAID accession number	Source	Amino acid Mutations*	Depositor	Notes
NR-52281	USA-WA1/2020	Jan 19, 2020	S	A	EPI_ISL_404895	Male in 30s with mild disease, returning traveler from Wuhan, China	NS8 L84S	Centers for Disease Control and Prevention, Atlanta, GA, USA	Standard strain used in multiple in vitro and in vivo SARS-CoV-2 studies
NR-52370	Germany/BavPat1/2020	Jan 28, 2020	G	B	EPI_ISL_406862	Mildly asymptomatic adult male from a Bavarian transmission cluster	Spike **D614G**	Drs. Roman Wölfel and Rosina Ehmann, Bundeswehr Institute of Microbiology, Munich, Germany	Also referred to as Germany/MUC-IMB1/2020 or Human/DEU/BavPat-ChVir929/2020. Spike mutation was associated with increased transmissibility.
NR-53514	New York-PV-08410/2020	Mar 16, 2020	GH	B.1	EPI_ISL_421374	Isolated from a nasal swab collected from a 63 year old male patient with a fatal respiratory illness in New York, USA	NSP2 T85I, NSP12 P323L, Spike **D614G**, NS3 Q57H	Dr. Adolfo García-Sastre, The Icahn School of Medicine at Mount Sinai Medical School, New York, NY, USA	Belongs to clade 'A2a', a large transmission cluster in New York City during a major outbreak in March 2020. It was phylogenetically similar to isolates from Europe.
NR-54008	hCoV-19/South Africa/KRISP-EC-K005321/2020	Nov 15, 2020	GH	B.1.351	EPI_ISL_678570	Isolated from an oropharyngeal swab from a 57-year-old human male in Harry Gwala district, KwaZulu-Natal, South Africa.	Spike A243del, A701V, D80A, D215G, **D614G**, **E484K**, K417N, L242del, L244del, **N501Y**; E P71L; N T205I; NS3 Q57H, S171L; NSP2 T85I; NSP3 K837N; NSP5 K90R; NSP6 F108del, G107del, S106del; NSP12 P323L	Dr. Alex Sigal, Africa Health Research Institute and Prof. Tulio de Oliveira, KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), Durban, South Africa	The deposited virus (after passage 3) harbored additional mutations compared to the clinical isolate: deletion in Furin cleavage site in Spike (677-681del), ORF1a (Q3878R)
NR-54011	USA/CA_CDC_5574/2020	Dec 29, 2020	GR	B.1.1.7	EPI_ISL_751801	Isolated from nasopharyngeal swab in San Diego, California, USA. Lineage first reported in the United Kingdom.	Spike A570D, **D614G**, D1118H, H69del, **N501Y**, P681H, S982A, T716I, V70del, Y145del; M V70L; N D3L, G204R, R203K, S235F, NS3 T223I; NS8 Q27stop, R52I, Y73C, A890D; NSP3 I1412T, T183I; NSP6 F108del, G107del, S106del; NSP12 P323L, A454V, K460R	Centers for Disease Control and Prevention, Atlanta, GA, USA	One additional SNP in ORF1ab L3826F was reported in the deposited passage two virus, in comparison to the clinical specimen.
NR-54008	hCOV-19/England/204820464/2020	Nov 24, 2020	GR	B.1.1.7	EPI_ISL_683466	Isolated from a 58-year old male from England, United Kingdom	Spike A570D, **D614G**, D1118H, H69del, **N501Y**, P681H, S982A, T716I, V70del, Y145del; N D3L, G204R, R203K, S235F, NS8 R52I, Y73C; NSP3 A1305V, I1412T, T183I; NSP6 F108del, G107del, S106del; NSP12 P323L; NPS13 K460R; NSP14 E347G	Dr. Bassam Hallis, Pre-Clinical Development at Public Health England, Salisbury, UK	Also referred to as UK/VUI/3/2020 isolate.

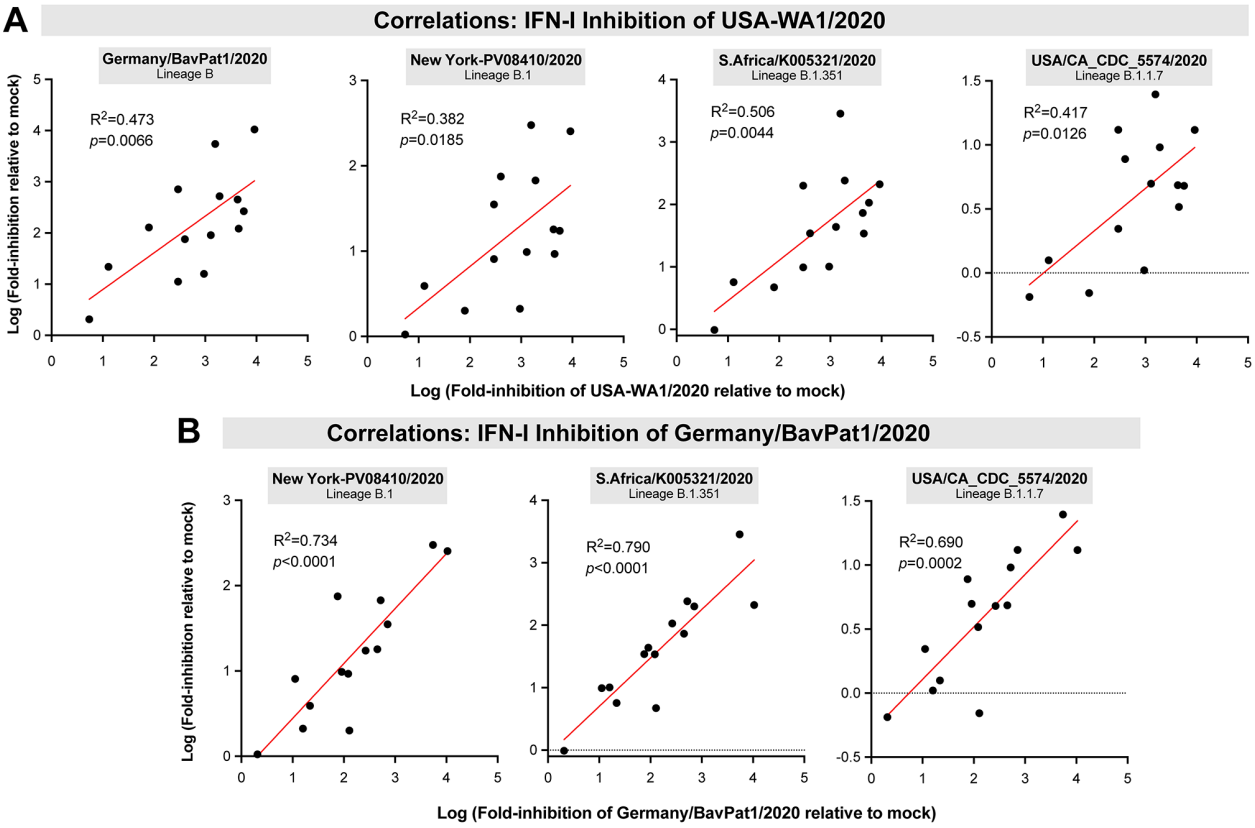
*Mutations were inferred relative to the reference hCOV-19/Wuhan/WIV04/2019 sequence (GISAID.org).



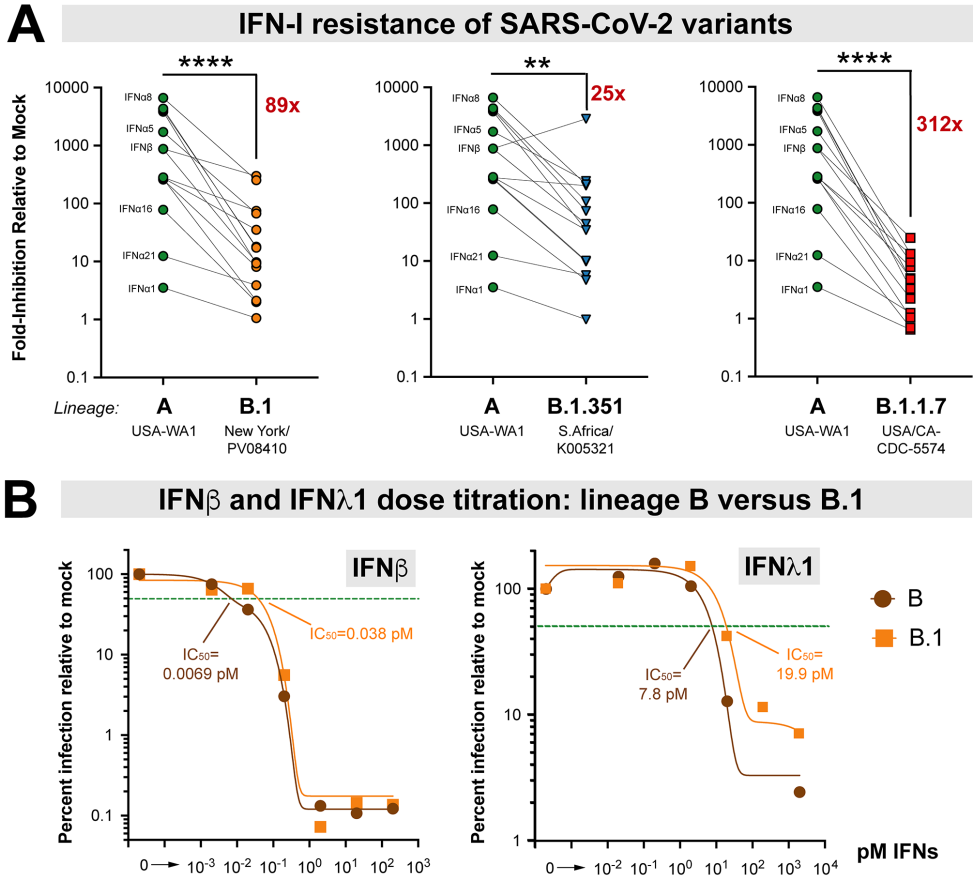
Supplementary Fig. 1 | Preparation of SARS-CoV-2 Isolates for IFN sensitivity assays. (A) SARS-CoV-2 stocks were obtained from BEI Resources. These original stocks were propagated in VeroE6 cells and titered to obtain 50% tissue culture infectious doses ($TCID_{50}$) per ml. Upon receipt, these original stocks were passaged and amplified once in A549-ACE2 cells. (B) Amplified virus stocks were titered in A549-ACE2 cells in a 48-well format for the subsequent inhibition assays. The pink line corresponds to the target virus copy number in untreated cells (~10^5 copies) and asterisks correspond to the approximate amount of virus that was used to reach that level for the inhibition assays. (C) Identification of an IFN dose for quantitative assessments of variant IFN-sensitivity. A ten-fold dilution series of IFNλ1 and IFNβ (0.002 to 2000 pM) were pre-incubated with A549-ACE2 cells for 24 h and USA-WA1/2020 RNA copies were evaluated by qPCR. Red dotted lines correspond to the dose (2 pM) that allowed for maximum discrimination of the antiviral potencies of the 2 IFNs. Points correspond to mean values and error bars correspond to standard deviation of triplicate measurements.



Supplementary Fig. 2 | Sensitivity of SARS-CoV-2 strains to IFN-I and IFN-III interferons. Viral RNA load data in Fig. 2 were normalized to mock (no IFN) as 100% and plotted in log-scale. Each dot corresponds to one of triplicate experiments, bars correspond to mean values and error bars correspond to standard deviations. The IFN sensitivity profiles were subdivided between isolates collected (A) early; and (B) later in the COVID-19 pandemic. Differences between the IFNs were evaluated using Friedman test. The *p*-values were noted in each graph. A post-hoc multiple comparisons test was performed for each IFN against the mock control; *, $p<0.05$, **, $p<0.01$.



Supplementary Fig. 3 | Correlation between IFN-I inhibition of different SARS-CoV-2 isolates. Fold-inhibition values relative to mock were calculated for each IFN-I tested then compared to (A) the USA-WA1/2020 strain and (B) the Germany/BavPat1/2020 strain. Log-transformed values were compared. Linear regression was performed in GraphPad Prism 8, with the best-fit line (red), R^2 and *p*-values indicated.



Supplementary Fig. 4 | IFN-I resistance of emerging SARS-CoV-2 variants. (A) Comparison of IFN-I sensitivities of lineage A versus B.1, B.1.351 and B.1.1.7. The mean fold-inhibition values relative to mock were compared for the 14 IFN-Is. The average fold-inhibition values are noted, and the graphs were plotted on a log-scale. Differences were evaluated using a nonparametric, two-tailed Wilcoxon matched-pairs signed rank test. ****, $p<0.0001$, **, $p<0.01$. (B) Dose-titration of IFNβ and IFNλ1 against lineage B (Germany/BavPat1/2020) versus B.1 (New York/PV08410/2020) isolates. A549-ACE2 cells were pre-treated with serial 10-fold dilutions of IFNs for 18 h in triplicate and then infected. Supernatants were collected after 24 h, SARS-CoV-2 N1 copy numbers were determined by qPCR, and then normalized against mock as 100%. Non-linear best-fit regression curves of mean normalized infection levels were used to interpolate 50% inhibitory concentrations (green dotted lines).